University of Gondar

College of Natural and Computational Science

Department of Chemistry

M.Sc. Thesis

Electrochemical Determination of Paracetamol Using Activated Glassy Carbon Electrode

By

Meselu Eskezia

Advisor: Dereje Yenealem (M.Sc.)

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Masters of Science in Chemistry/Physical Chemistry

Gondar, Ethiopia

June, 2017

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Thesis Approval Sheet

The Thesis entitled 'Electrochemical Determination of paracetamol using activated glassy carbon electrode' by Meselu Eskezia is approved for the degree of Master of Science in chemistry/physical chemistry.

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List of abbreviations and symbols

AGCE	Activated Glassy Carbon Electrode
CV	Cyclic Voltammetry
DPV	Differential Pulse Voltammetry
DP	Differential Pulse
GC	Glassy Carbon
GNMCPE	Gold nanoparticles modified carbon paste Electrode
GMCPE	Graphene modified carbon paste electrode
E	Potential
E^0	Formal potential
E_{pa}	Anodic peak potential
E_{pc}	Cathodic peak potential
F	Faradays constant
I_p	Peak current
LOD	Limit of Detection
LDR	Linear Dynamic Range
m	Slope of calibration curve
NAPQI	N-Acetal-P-Quinoneimine
NHCFMCPE	Nickel Hexacyano ferrate modified carbon Paste electrode
NPV	Normal Pulse Voltammetry
R	Ideal gas constant
RSD	Relative standard deviation
SWF	Square Wave Frequency

SPGrE	Screen printed graphene electrode
SWA	Square Wave Amplitude
PAR	Paracetamol
PEDOT/GC	Glassy carbon electrode modified with Poly (3, 4- Ethylene dioxythiophene
PBS	phosphate buffer solution
Pka	Power dissociation constant
UA	Uric Acid
δ	Standard deviation
Γ^*	Surface adsorption
α	Electron transfer coefficient

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Abstract

In this study the electrochemical property of paracetamol (PAR) was investigated at a glassy carbon electrode and activated glassy carbon electrode. Cyclic voltammetry and differential pulse voltammetry were used as diagnostic techniques in the determination of paracetamol. The activated glassy carbon electrode was prepared by activating 200 s in a time base technique at a potential of 1750 mV. The AGCE exhibited excellent electro-catalytic behaviour towards PAR which confirmed by the enhancement of both peak currents peak potential shifts to less positive values by (13 mV) in comparison with GCE. The electrode process of paracetamol was studied and some the experimental parameters which affect the response paracetamol, such as pH, effect of PAR concentration and scan rate on AGC electrode. The analysis of cyclic voltammogram gave fundamental electrochemical parameters including electron transfer coefficient (α) and the heterogeneous rate constant (k_s). The variation of scan rate study shows that the system undergoes adsorption controlled process. The equation of the calibration curve was found to be: I_p (μA) =0.429C (μM) + 6.43, R^2=0.993. The LOD and LOQ for this study were determined to be 8×10^{-8} mol L^{-1} and 2.6×10^{-7}mol L^{-1} respectively. The degree of recovery for paracetamol was calculated by adding the standard solution of PAR to paracetamol tablets and the results were found to be 105%. The effects of some interfering substances in the determination of paracetamol were also studied and their interferences were found to be negligible which provided the selectivity of the activated electrode.

***Key words**: paracetamol, activated glassy carbon electrode, cyclic voltammetry, differential pulse voltammetry, interference.*

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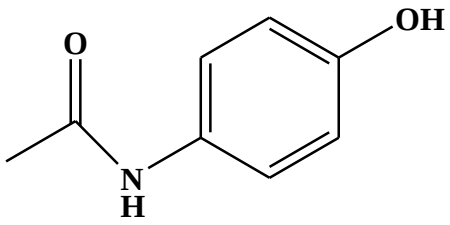
1. INTRODUCTION

1.1. Background of the study

Drug control has been on the global agenda for more a century in the world. So, drug analysis is an important tool for drug formulations which has great impact on public health. Hence, the development of simple, sensitive and rapid method to determine the active ingredients in drugs seems essential [1]. From the environmental point of view, pharmaceuticals including antibiotics are a new group of manmade chemicals of concern entering the environment at concentrations such that their health effects are unknown. So, paracetamol is one of the antibiotic drugs that used to fight infections caused by bacteria or other microbes [2].

Paracetamol, N-(4-hdroxyphenyl) acetamide is a widely used analgesic and antipyretic drug [3]. It is one of the most popular and widely used drugs for the treatment of pain and reduction of fever. It occupies a unique position among analgesic drugs [4]. Generally, paracetamol does not exhibit any harmful side effects due to its rapidly and completely metabolized. However, the overdose of paracetamol can lead to the accumulation of toxics metabolites, which may cause liver disorder, kidney damage, skin rashes and inflammatory of the pancreas [5]. It is commonly used for the relief of headaches, other minor aches, pains and a major ingredient of in common cold [6]. Paracetamol described as 4-hydroxyacetanlide or N- acetyl-p-aminophenol is known as acetaminophen [7] and its chemical formula, $C_8H_9NO_2$ and its structure is as shown schemes,



Scheme 1. Chemical structure of paracetamol.

Paracetamol is an analgesic drug which is extensively used to alleviate headaches, backache and postoperative pain. Paracetamol was first synthesized in the late nineteenth century and it is considered to possess analgesic and antipyretic properties. Paracetamol is an acylated aromatic amide that has been in use as an analgesic for home medication for over 50 years, and is accepted as an effective drug for the relief of pain and fever in adults and children [8].

The primary hydrolytic degradation of paracetamol is p-aminophenol, which can be present in pharmaceuticals preparation as a synthetic intermediate or as a degradation product of paracetamol that can cause serious nephrotoxocity and tetragenic effects [9, 10]. Paracetamol acts as painkiller by inhibiting prostaglandin's synthesis in the central nervous system and relieves fever by sedating hypothalamic heat-regulating center. It is mainly used as an alternative to aspirin for relief of mild pain and antipyretic. Paracetamol is as an active ingredient in pharmaceutical preparations and can cause serious or fatal adverse effects when taken in overdose; the liver conjugation becomes inundated, causing paracetamol to be metabolised by an alternative pathway [11].

Paracetamol, a weak acid having p*K*a value 9.5, rapidly gets absorbed and distributed after oral administration and is easily excreted in urine [12, 13]. Taking high doses of Paracetamol may cause adverse effects in the body but, in proper doses it does not display any side effects. Nowadays, Paracetamol is widely used for its remarkable therapeutic characteristics thus precise determination and control of its quality is very important [8]. The development of simple, sensitive and accurate electroanalytical methods for the determination of paracetamol is very important. The various techniques have been employed for the determination of paracetamol in the body fluids and pharmaceuticals preparations including spectroscopy, chromatography, titrimetry and chemiluminscence [8, 11, 12, 14].

However, most of these techniques suffer from some disadvantages like; high cost, require extraction process, long analysis time, requirement for sample pre-treatment which is time consuming manipulation steps [15], need special training, portable [16**]**, sophisticated instrument and making them unsuitable for routine analysis [17] and also these methods usually involves hydrolysis of paracetamol sample to 4-aminophenol, which the required the formation of a colored complex using an appropriate reagent, which takes a long time to perform [9].

On the other hand electrochemistry offers a number of very attractive advantages such as low cost, easy to manipulate, portable and fast. It has been widely employed in biological matrixes, pharmaceutical and some drugs containing tertiary amine functional group, due to its continuance, sensitivity, reproducibility and selectivity towards many target analytes [19].

An electrochemical technique such as voltammetry is used for the determination of paracetamol that offers high sensitivity, low cost and accuracy beside wide linear range. Paracetamol is an electroactive compound (contains hydroxyl and NH groups on its aromatic rings) [10] and can be oxidized under suitable conditions, the use of electrochemical detection can be considered appropriate due to its rapid response and high sensitivity. Furthermore, because of the potential for miniaturization, ease of manufacture and low cost, electrochemical techniques can be considered as an attractive method for the determination of paracetamol [39].

Many papers have been published about the electrochemical determination of paracetamol based on its oxidation behaviour with different electrodes such as, C_{60}-modified glassy carbon electrode [12], Poly (4-vinyl pyridine) multi walled carbon nanotubes modified glassy carbon electrode [8], glassy carbon electrode [13], screen printed graphene electrode [19], gold nanoparticles electrodes [10], Bismuth oxide modified glassy carbon electrode [21] and Ni- modified electrode [39]. These reports showed good detection limits and sensitivity but, the main drawback is the need of extra time through the consuming modification process which usually involves several steps to incorporate the modifier to the substrate and also the costs **[**19].

In this study, no study has been reported for determination of paracetamol using activated glassy carbon electrode. Activated glassy carbon-based electrodes usually have a wider potential range than the other solid electrodes because of their broad potential window, low background current; chemical inertness, low cost and suitability for various sensing and detection applications. However, electron transfer rates observed at carbon surfaces are often slower than those observed on noble metal electrodes [6].

This paper describes the use of a simple electrochemically activated glassy carbon electrode without employing any expensive modifier for determination of paracetamol using 0.1mol L^{-1} phosphate buffer solution (pH 7.0).

1.2. Statement of the problem

In the world, drug has played a significant role in treating health problems in human being. But the level of drug is not well studied by the drug quality control especially in Ethiopia. This inhibits the standardization and internationally recognized system of the drug in Ethiopia. The main thing is the quality of all drugs are not studied and identified by drug quality control in pharmaceuticals formulations. The other important concern is also the chemical composition of the drugs are not well known to use appropriately and difficult to regulate the dose and the concentration of the drug. These posses the use of drugs is a risk of toxicity and deaths to the users. Therefore, this study aims at determining the levels of PAR and to regulate the dose and the concentration of the drugs as a part of the solution to challenges the problems by developing cost effective valid electrochemical methods.

1.3. Significance of the study

The results of this study will be provide information about the use of PAR in human is limited by its toxicity and its use in developed countries is limited to topical application for the treatment of pain and reduction of fever. However, the use of PAR was found primarily in developing countries due to its low cost. Thus, evaluating and monitoring the level of PAR are necessary to ascertain that the drug should not be misused and does not cause a danger to human health. This research will be signify to; create awareness in the users about the impact of PAR and it can be used as the starting materials for others who want to search further about PAR as well as similar analgesic drugs.

1.4. Objective of the study

1.4.1. General objective

The general objective of this work relies on develop the electrochemical sensor based on potentially activated electrode for sensitive and selective determination of paracetamol using AGCE.

1.4.2. Specific objective

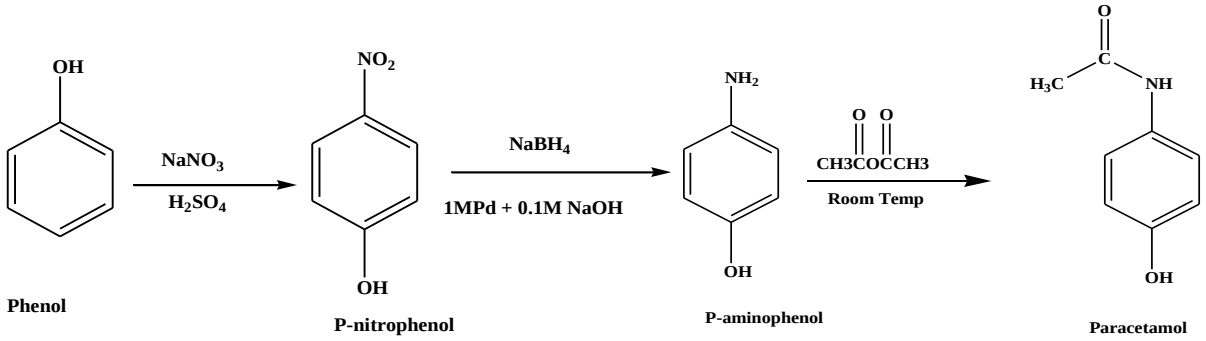
The specific objectives of this study are to:

- Investigate the electrochemical behaviours of PAR by using GCE and AGCE.
- Investigate the effect of pH and scan rate on the electrochemical response of PAR at AGCE
- Determine electron transfer coefficient, number of electrons (n), number of protons (m), surface adsorption (Γ^{*}) and limit of detection (LOD) of PAR using AGCE.
- Compare the results of this study with results of other studies
- Distinguished the type of reaction is whether diffusion or surface adsorption controlled.
- Study the effects of interference on the determination of PAR using AGCE.

2. LITERATURE REVIEW

2.1. Synthesis of paracetamol

Paracetamol is antipyretic drugs used in medicine. It is a mild painkiller and reduces the temperature of patients with fever. These actions are known respectively as analgesic and antipyretic. In laboratory, paracetamol is easily prepared by nitrating phenol with sodium nitrate, separating the desired p-nitro-phenol from the ortho- by product and reducing the nitro group with sodium borohydride. The resultant p- aminophenol is the acetylated with acetic anhydride to paracetamol. The preparation of paracetamol from phenol can be three steps, the first step is nitration of phenol to give nitro-phenol, the second step is the reduction of nitro group to amine and the third step is formation of an amide (paracetamol) [7].



Scheme 2. Proposed synthesis of paracetamol from phenol.

2.2. Physical properties of paracetamol

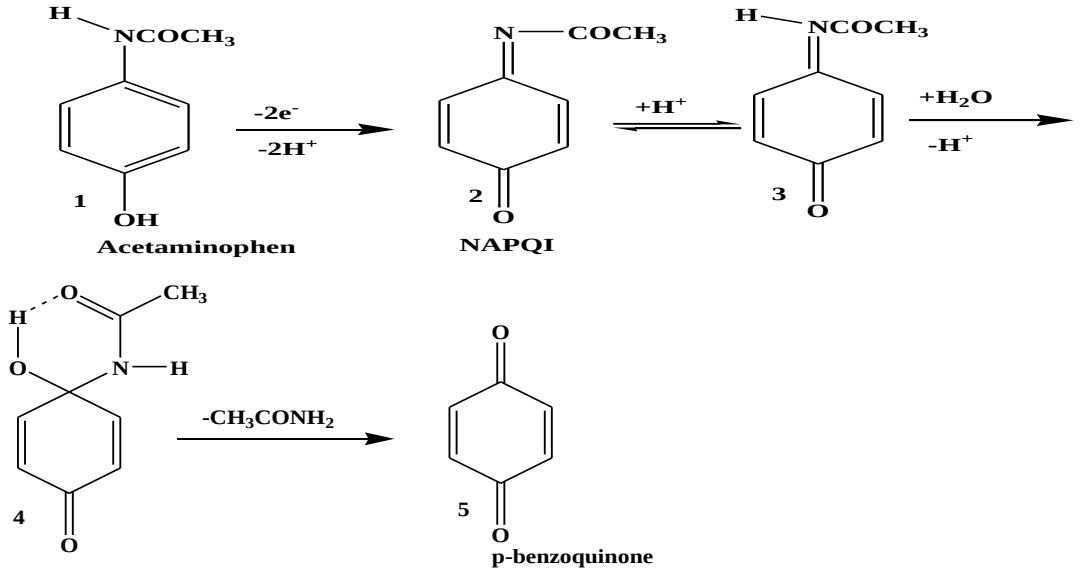
Paracetamol is a white, slightly odorless crystalline compound with a better taste [6]. It is soluble in organic solvents such as methanol and ethanol but slightly soluble in water and ether. The melting point of this compound is 169-170 ^{0}C [7].

2.3. Chemical properties of paracetamol

Paracetamol is most stable at saturated aqueous solutions. Its pKa value is 9.5 in aqueous media. The stability decreases in acid or alkaline conditions. Chemically N-(4 hydroxyl phenyl) acetamide is derived from the interaction of p-aminophenol and an aqueous solution of acetic anhydride [7].

2.4. Mechanism of the electrochemical oxidation of paracetamol

The cyclic voltammogram of the oxidation of paracetamol is characterized by an anodic peak in the positive going scan and the presence of cathodic peak on the reverse scan indicates that the electrochemical oxidation of paracetamol is reversible. The electrochemical oxidation mechanism of paracetamol proceeds by a two electron, two proton processes, and the result is N-acetyl-p-quinoneimine and the final product is p-benzoquinone [19].



Scheme 3. Mechanism of electrochemical oxidation of paracetamol**.**

Paracetamol is oxidized in pH dependent step involving the loss of two electrons and two protons to give N-acetyl-p-quinoneimine as shown in above step (1) mechanism. Under more acidic conditions, NAPQI is readily protonated to give species (3), which is a less stable, but electrochemically active species. Species (3) then rapidly yields a hydrated species (4), which is electrochemically inactive in the examined potentials. Finally, under increasingly more acidic conditions the hydrated species (4) converts to benzoquinone (5). Only under extremely acidic conditions was the reduction of benzoquinone observed with cyclic voltammetry **[**20].

2.5. Electrodes used in electrochemical studies of paracetamol

Electrochemical measurements are made in an electrochemical cell consisting of two or more electrodes and the electronic circuit for controlling and measuring the current and potential. In this study the electrochemical cells consists of three electrodes such as working, reference and auxiliary electrodes which are immersed in the sample solution. The working electrode is an electrode at which the reaction of interest occurs. The electrode is sensitive to the analytes concentration is called working or indicator electrode. There are different working electrodes such as mercury, gold, platinum and carbon **[**21].

In this study the working electrode is glassy carbon electrode because of its excellent mechanical and electrical properties, wide potential window chemical inertness (solvent resistance) and relatively reproducible performance. The second electrode is a reference electrode provides a stable and reproducible potential (independent of the sample composition), against which the potential of the working electrode is compared and whose potential remains constant [22]. The reference electrode in this study is Ag/AgCl electrode. The third electrode is counter or auxiliary electrode completes the electrical circuit and provides a reference potential against which we measure the working electrodes potential. The auxiliary electrode is platinum wire [21.

2.5.1. Activated glassy carbon electrode (AGCE)

Due to its physical and chemical properties, glassy carbon is become an interesting and widely applied electrode material in electrochemical techniques. It exhibits rather lower oxidation rate and high chemical inertness which, together with very small pore sizes and a small gas and liquid permeability, make glassy carbon a convenient inert electrode. Carbon-based electrodes are nearly ubiquitous in the laboratory today because of their availability in various forms and shapes, and usefulness over a wide potential range [22].

The most commonly used carbon-based electrode in the analytical laboratory is glassy carbon (GC). It is made by pyrolyzing a carbon polymer, under carefully controlled conditions, to a high temperature like 2000 ^{0}C. The electrochemical properties of GCE are related to the composition and the structure of the surface. Electrochemically activated GCE usually gives reproducible surface and improve electron transfer [23].

Glassy carbon is an inert material and when polished has a rather low surface area but it is porous and contains different functional groups [24].There are different pre-treatments and for preparing and activating the glassy carbon electrode surface for electrochemical measurements. Fresh and well- defined electrode surface can be prepared mechanical treatment, which involves abrasion with emery paper and polishing with alumina. Other methods such as laser treatment, irradiation of glassy carbon with ultrasound and activation glassy carbon using a carbon arc have been applied to create a reproducible and active glassy carbon electrode surface. However, it seems that the electrochemical treatment of glassy carbon electrodes is the most often used for this purpose [22].

Generally, the main reason for focusing the activation of glassy carbon electrode is that it is a cheaper electrode material than the noble metals, not time consuming to activate, no need of modification steps and it has lower background current, noise levels than other carbon materials and lower detection limit to analytical investigation [25].

2.6. Electrochemical techniques

Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as large linear dynamic range, with relatively low-cost instrumentation. After developing more sensitive pulse methods, the electroanalytical studies are more regularly used on the drug analysis in their dosage forms and especially in biological samples. However, electroanalytical techniques can easily solve many problems of pharmaceutical interest with a high degree of accuracy, precision, sensitivity, and selectivity employing this approach [26].

Voltammetry is an electrochemical technique based on the measurement of current that result from the application of potential. It is a typically performed using three electrode electrochemical cell. The use of three electrodes (working, auxiliary and reference) along with the potentiostat instrument allows accurate application of potential functions and measurement of the resultant current [27].The common characteristics of all voltammetric techniques is that involve the application of a potential (E) to an electrode and the monitoring of the resulting current (*i*) flowing through the electrochemical cell. All voltammetric techniques can be described as some function of *E*, *i*, and *t* [28].

2.6.1. Voltammetric techniques and their theoretical aspects

The different voltammetric techniques that are used to distinguished from each other primarily by the potential function that is applied to the working electrode to drive the reaction, and by the materials used as the working electrode [27].

2.6.1.1. Cyclic voltammetry

Cyclic voltammetry (CV) is the most versatile electroanalytical technique for the study of electroactive species. Its versatility combined with ease of measurement has resulted in extensive use of CV in the field of electrochemistry, inorganic chemistry and organic chemistry [29]. Cyclic voltammetry is a rapid voltage scan technique in which the direction of voltage scan is reversed [26].

The primary advantages of CV is it gives insight into both the half reactions takes place at the working electrode, provided at the time information about the chemical and physical phenomena coupled to the studied electrochemical reaction. The main instrumental parameter in the cyclic voltammetry is the scan rate, since it controls the timescale of the voltammetric experiment. A cyclic voltammogram readily shows the presence of species in that can undergo redox reactions at the electrode within the applied potential range [26, 29]. As shown **Fig.1**, the most important informations a cyclic voltammogram are the cathodic peak potential (E_{pc}), anodic peak potentials (E_{pa}), anodic peak current (i_{pa}) and the cathodic peak current (i_{pc}) [27, 30].

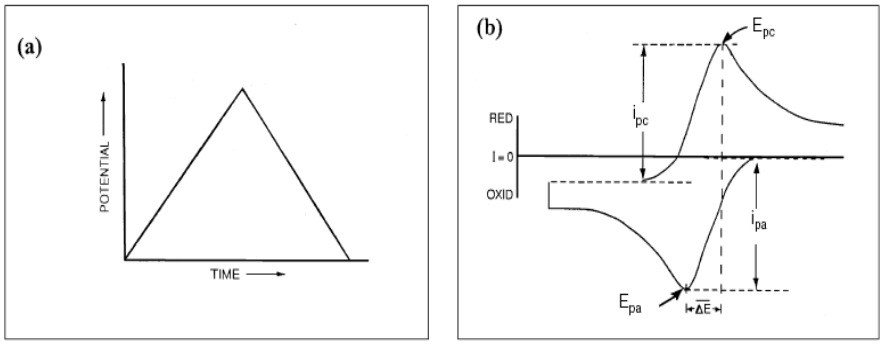


Fig. 1. (a) Excitation signal, (b) response obtained for the reversible by cyclic voltammetry.

If the electrons transfer process is fast compared with other process (such as diffusion), the reaction is said to be electrochemically reversible, and the peak separation is,

$$\Delta E_p = E_{pa} - E_{pc} = \frac{2.303RT}{nF} \quad (1)$$

Thus, for a reversible redox reaction at 25 °C with n electrons ΔE_p should be 0.0592/n V or about 60 mV for one electron. In practice this value is difficult to attain because of such factors as cell resistance. Irreversibility due to a slow electron transfer rate results in $\Delta E_p >$ 0.0592/n V, greater than 70 mV for a one-electron reaction [28]. The formal reduction potential (E^0) for reversible couple is given by,

$$E^0 = \frac{E_{pa} + E_{pc}}{2} \quad (2)$$

2.6.1.2. Application of cyclic voltammetry

A CV is obtained by measuring the current between the working and the counter electrode as a function of the potential (normalized to the potential of the reference electrode) [31]. CV is a very popular technique for electrochemical studies of new systems, and sensitive tool for obtaining information about fairly complicated electrode reactions. CV methods have found to have extensive applications for the evaluation of thermodynamic and kinetic parameters such as number of electrons change, heterogeneous rate constant, entropy, Gibb's free energy and diffusion coefficient etc., of a number of redox reactions and associated chemical reactions. These methods are especially useful in both oxidation and reduction process and to study the multiple electron transfer in an electrochemical reaction [27].

2.6.2. Square-wave voltammetry (SWV)

SWV is a powerful electrochemical technique that can be applied in electroanalytical measurements. Square-wave voltammetry (SWV) is the most advanced and the most sophisticated technique in the family of pulse voltammetric techniques. The net current in SWV is obtained as a subtraction between the forward and the backward currents in **Fig.2**, [30]. Among the various voltammetric techniques, exceptional versatility is found in a method called square wave voltammetry. SWV has several advantages. Among these are its excellent sensitivity, excellent peak separation [27] and the rejection of back ground currents.

Another is its speed [28], high effective scan rate, reducing scan time. Because of this advantage; SWV is employed more often than other pulse technique [32].

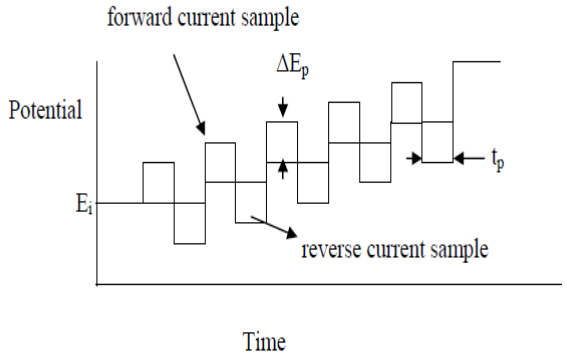


Fig. 2. The applied potential waveform in square wave voltammetry.

2.6.3. Differential pulse voltammetry (DPV)

DPV is an extremely useful technique for measuring trace levels of pharmaceutically active compounds [32]. This technique was proposed by Barker and Gardner. DPV can provide greater sensitivity and more efficient resolution and differentiation of various species [27]. DPV technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential [28].

The excitation waveform is basically the staircase. In DPV technique, fixed-magnitude pulses superimposed on a linear potential ramp are applied to the working electrode at a time just before the end of the drop. The difference between these two current values as a function of the potential is recorded and displayed. The application of these pulses allows for discrimination of the unwanted capacity current from the required faradic current [32].

The differential pulse voltammogram is thus, a plot of current difference versus applied potential. The use of pulse is minimizes the effect of charging current as in normal pulse voltammetry. The pulse height is a parameter that can be varied in differential pulse voltammetry to improve sensitivity. As the pulse height increase, the peak potential increase in an almost linear manner [35].The greatest advantage of DP method is increased sensitivity, allowing low value of LOD of various compounds [32].

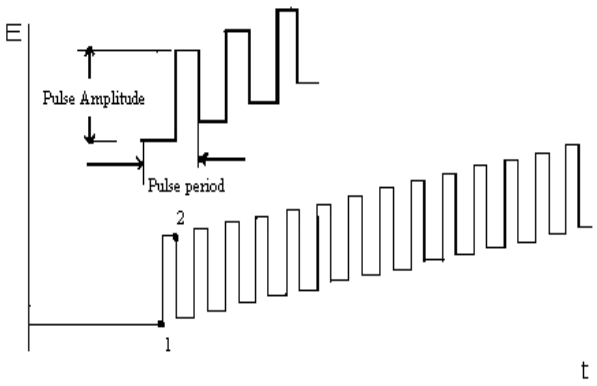


Fig. 3. Excitation waveform of differential pulse voltammetry**.**

2.6.4. Supporting electrolyte in electrochemical study of paracetamol

Electrochemical investigations consists of a supporting electrolytes that required in controlled potential experiments to decrease the resistance of the solution, to eliminate the electro migration effects and to maintain a constant ionic strength(swamping out the effect of variable amounts of naturally occurring electrolyte). The inert supporting electrolytes include an inorganic salt, a mineral salt or a buffer solution. However, for the determination of paracetamol in this study the supporting electrolyte is a buffer system. Buffer systems such as acetate, phosphate and citrate are used when a pH control is essential [40]. In this work the supporting electrolyte is a phosphate buffer with pH 7.0. The supporting electrolyte should be prepared from highly purified reagents and should not be easily oxidized or reduced.

2.6.5. Solvent used in electrochemical study of paracetamol

Electrochemical measurements are commonly carried out in a medium that consists of a solvent containing a supporting electrolyte**.** The choice of the solvent is dictated primarily by the solubility of the analyte and its redox activity and by solvent properties such as the electrical conductivity, electrochemical activity and chemical reactivity**.** The solvent should not react with the required analyte and should not undergo electrochemical reactions over a wide potential range [41]. In this study the solvent and the supporting electrolytes are phosphate buffer solution pH 7.0.

3. EXPERIMENTAL PARTS

3.1. Instrumentation

The electrochemical experiments were carried out in a three electrode systems containing Ag/AgCl as a reference electrode, platinum wire as a counter electrode, bare glassy carbon electrode and activated glassy carbon electrode as a working electrode. The experiment and processing of data were made using CHI760E electrochemical workstation, CH Instrument (Inc., USA), which was connected to a Dell desktop computer with conventional three electrode configuration. The pH of all solutions was measured with a JENWAY model 3510 digital pH meter with a combination glass electrode. Digital balance was used for mass measurements. The experiments were conducted in phosphate buffer solution at pH 7.0 and at room temperature. Cyclic voltammetry and differential pulse voltammetry were used for this study.

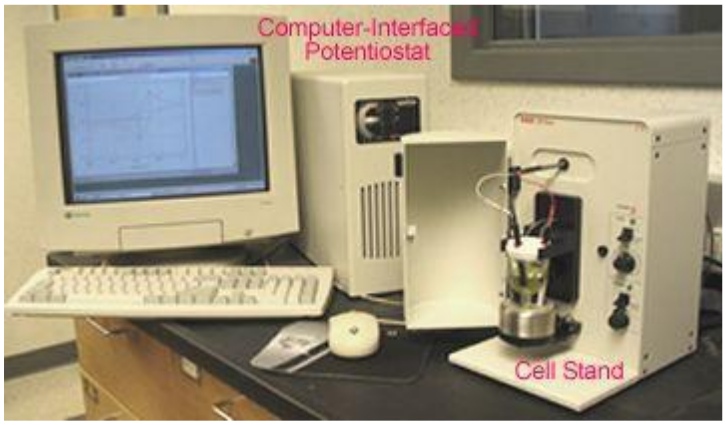


Fig. 4. Cyclic voltammetry instrument (including potentiostat).

3.2. Chemicals and reagents

Standard paracetamol (Addis pharmaceuticals factory, Ethiopia), anhydrous di potassium hydrogen orthophosphate (BDH, England), potassium di hydrogen phosphate (Sigma-Aldrich, Switzerland), sodium hydroxide (BDH, England), uric acid (LABORT, India) sulphuric acid and paracetamol tablets (EPHARM) were used in the experiment without any purification. The stock solution of paracetamol was prepared and stored in a refrigerator until used. An aqueous solution was prepared daily of the working days by the dilution of the stock solution with phosphate buffer pH 7.0. Phosphate buffer solutions (0.1 M KH_2PO_4 and

K_2HPO_4) were prepared by using distilled water. Distilled water was used throughout the experiment. All chemicals were of analytical grade.

3.3. Preparation of activated glassy carbon electrode

Before activation, the surfaces of glassy carbon electrode (3mm diameter) was polished to mirror with alumina slurry with a polishing pad and then thoroughly rinses with distilled water. The cleanness of the electrode was checked by a 0.5 M sulfuric acid by running in cyclic voltammetry with a potential window between -800 mV - 800mV at a scan rate of 100 mV s^{-1}. Then the GC electrode was activated for 200 s in a time base technique at a potential of 1750 mV in 0.1 M KH_2PO_4 and K_2HPO_4 phosphate buffer solution at pH 7.0 and the GC electrode was activated by running cyclic voltammetry from 0.0 to 700 mV for six cycles. The activated electrode was run in cyclic voltammetry until the voltammogram was stable.

3.4. Preparation of phosphate buffer and standard solutions of the analytes

For all of the experiments, a mixture of 0.1M K_2HPO_4 and 0.1M of KH_2PO_4 buffer solution (pH 7.0) was used. Concentrated NaOH and HCl solutions were used to adjust the pH of the buffer solutions. Stock solution of paracetamol (1mM) prepared by dissolving 0.075 g of paracetamol in PBS of pH 7.0. The required amounts of paracetamol working solutions were prepared by diluting the stock solution with phosphate buffer solution supporting electrolyte (pH 7.0). Standard solutions in tablet were prepared by spiking of the drug in to an aqueous stock solution of standard paracetamol samples. The stock solution of uric acid was prepared by diluted with phosphate buffer solution.

3.5. Sample preparation from tablets

Ten tablets were purchased from commercially available pharmaceuticals drug shops (500 mg paracetamol per tablet). Five tablets were accurately weigh using digital balance and finely powder in mortar and pestle. Then an adequate amount of powder which was equivalent to the standard powder was weighed and added into 100 volumetric flasks and diluted with a pH 7.0 phosphate buffer solution. Then the flask was thoroughly shaken until sample dissolved and the mixture was filled with the buffer solution.

3.6. Electrochemical measurements

Electrochemical determination of paracetamol was carried out in a voltammetric cell 0.1 M PBS (pH 7.0) as a supporting electrolyte solution. The electrochemical behaviour of paracetamol at AGC electrode was investigated using cyclic voltammetry. The determination of paracetamol was carried out by using differential pulse voltammetry (DPV) by scanning the potential in the range from 0.0 to 500 mV at the pulse amplitude of 50mV and pulse repeat time of 0.5 seconds were used. The paracetamol concentrations were obtained by measuring the heights of the oxidative peak currents. The detection limit was calculated as three times the standard deviation of phosphate buffer solution of paracetamol divided by the slope of the calibration curve and limit of quantification was calculated as ten times of the standard deviation phosphate buffer solution of paracetamol divided by the slope of the calibration curve.

4. RESULTS AND DISCUSSION

In this study the electrochemical oxidation of paracetamol has been studied using cyclic voltammetry and differential pulse voltammetry.

4.1. Cyclic voltammetric investigation of PAR using bare and AGCE

The electrochemical behaviour of PAR was examined using cyclic voltammetry at a scan rate of 100 mV s^{-1}. **Fig**. 5 shows typical voltammogram of 0.1mM of PAR, in phosphate buffer solution pH 7.0 at a scan rate of 100 mV s^{-1} recorded at two different working electrodes (i,e. bare GC and AGCE). At bare GC electrode, Paracetamol shows irreversible behaviour with relatively weak redox current peaks with high peak potential difference (ΔE_p=0.450 V and slow electron transfer behaviour on bare GC electrode [9].

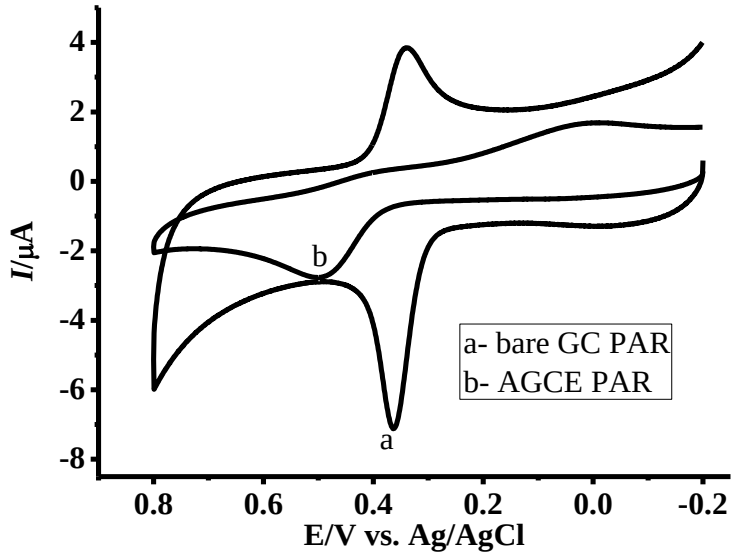


Fig. 5. Cyclic voltammogram of 0.1mM of PAR obtained at bare GCE (curve a) and AGCE (curve b) in 0.1 M PBS at pH 7.0 with scan rate of 100 mV s^{-1} (Background subtracted).

In comparison to unactivated (bare) GC electrode, electrochemical response of paracetamol at activated glassy carbon electrode after activated the electrode shows both cathodic and anodic peak current with significantly increment with reducing the overpotential by 0.130V. This indicates activation of electrode alters both the electrochemical oxidation and reduction of paracetamol from irreversible to reversible reaction.

The peak-to-peak separation of PAR at activated glassy carbon electrode (ΔE_p = 0.025V) is much smaller than that of the bare GC electrode [10]. The ratio of redox peak current (I_{pa}/I_{pc}) was 1.85, which shows the characteristics of irreversible electrode process. However for reversible electrode reaction the ratio of I_{pa} to that of I_{pc} is one [7].

Activated GC electrode shows a fast electron transfer rate (kinetics) due to good conductivity and a large capacitive current of the electrode. This indicates that the activated glass carbon electrode shows electro-catalytic activity towards paracetamol. To generalized the above discussion the catalytic properties of activated glassy carbon electrode surface caused decrease the over potential of oxidation reaction, increase the sharpness of both cathodic and anodic peak current and the reversibility of electron transfer process [19].

Table 1. Peak current and peak potential of PAR on bare and activated GC electrode.

Analytes	Bare GC electrode					Activated GC electrode				
PAR	Peak current (μ A)		Peak potential (V)		Δ E (V)	Peak current (μ A)		Peak potential (V)		Δ E (V)
	I_{pa}	-2.71	E_{pa}	0.479	0. 450	I_{pa}	-7.07	E_{pa}	0.36	0.02
	I_{pc}	1.607	E_{pc}	0.2 9		I_{pc}	3.78	E_{pc}	0.34	

4.2. Effect of operational parameters

4.2.1. Effect of solution pH

In order to optimize the response of activated GC electrode for paracetamol oxidation, the effect of pH on the electrochemical oxidation was investigated by cyclic voltammetry technique at different pH using phosphate buffer solution with a pH range of 4 - 10 at a scan rate of 100 mV s^{-1} to determine its effect on the catalytic oxidation of 0.1mM paracetamol at activated GC electrode. As shown **Fig. 6**, the pH of the solution obviously influenced the potential and the currents of both cathodic and anodic peaks of paracetamol.

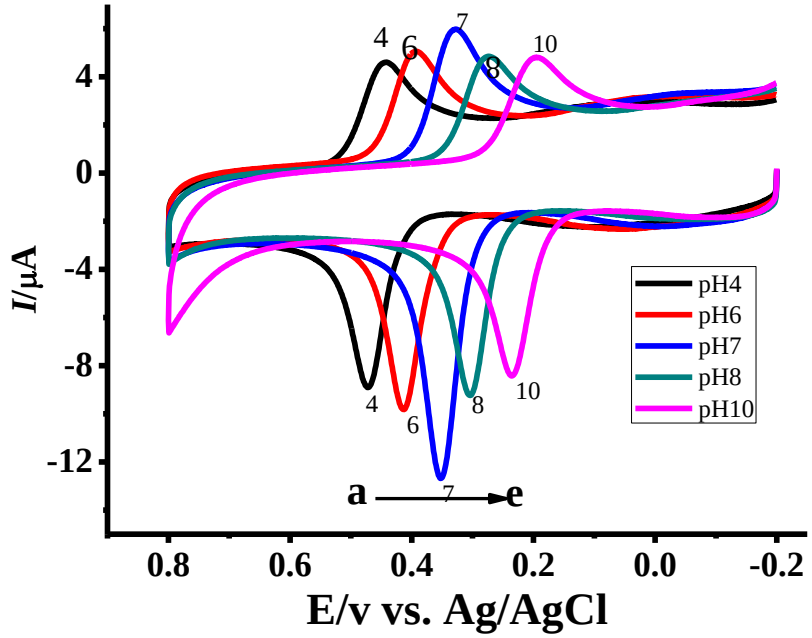


Fig. 6. Cyclic voltammogram of 0.1 mM of PAR in 0.1 M PBS at different pH values (4, 6, 7, 8 and 10) with a scan rate of 100 mV s^{-1} using AGCE (Background subtracted).

As shown in **Fig. 7**, the peak currents increased with increasing pH up to 7.0 and the peak currents slowly decreased from 7-10 (i,e higher pH values), suggested that the oxidation of paracetamol is kinetically less favourable at higher pH values. In addition, **Fig. 8** also shows the relationship between the peak potential of paracetamol and the pH value. As can be seen, both oxidation potential (E_{pa}) and reduction potential (E_{pc}) shift to negatively direction with the increase of pH from 7.0 to 10. This observation is strong evidence that reflects the involvement of protons in the electrode process [39]. Furthermore, the potential was shifted to the direction of more negative potentials with increasing pH values, i.e. to the lower potential, suggesting that the ease of oxidation the protonated molecules [40]. The better sensitivity and shapes of voltammogram (maximum peak current) was observed at pH 7.0 suggested it as optimal pH value.

Generally, the electrochemical oxidation of paracetamol at activated glassy carbon electrode is pH dependent. At a pH near to 7.0 N-acetyl-p-Quinoneimine exists in its stable and unprotonated form [7]. If the pH was higher than 7, the drugs inclined to decompose, resulting in the decrease of the response. As can be seen, the peak potential for paracetamol oxidation varies linearly with. Therefore, pH 7.0 was better for further analysis.

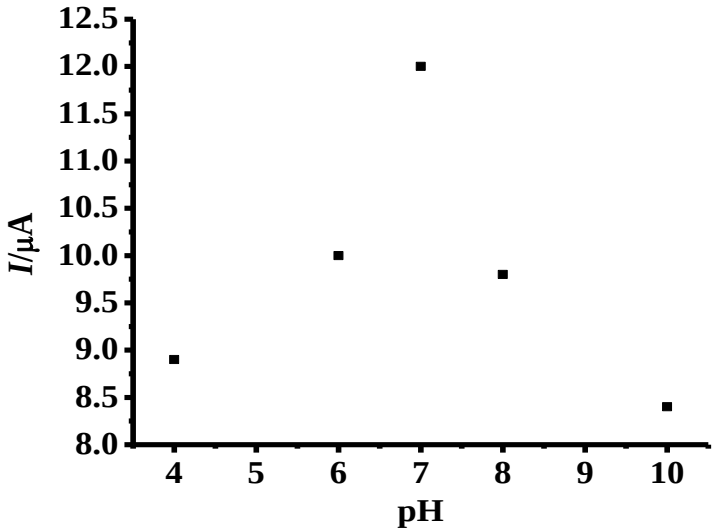


Fig.7. plot of peak current (I_{pa}) versus pH at a scan rate of 100 mV s^{-1}.

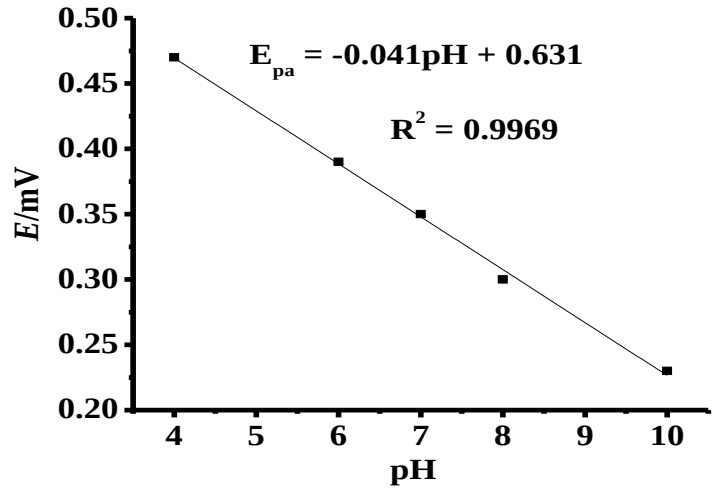


Fig. 8. Plot of peak potential (E_{pa}) versus pH at a scan rate of 100 mV s^{-1}.

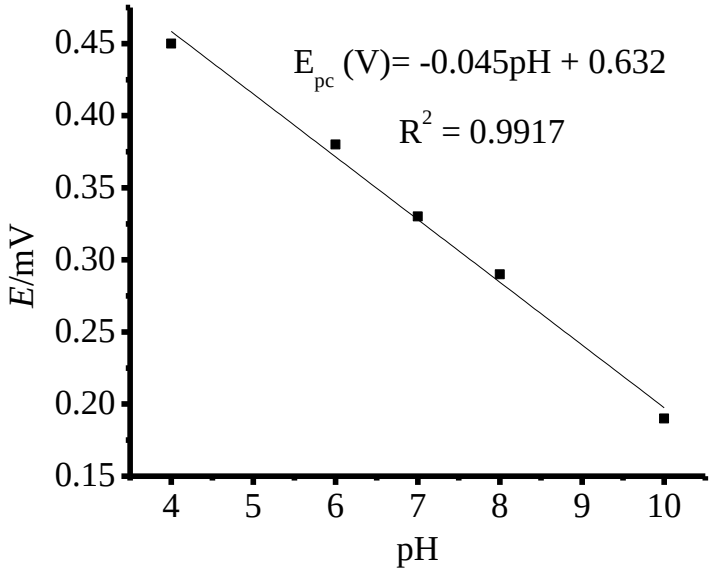
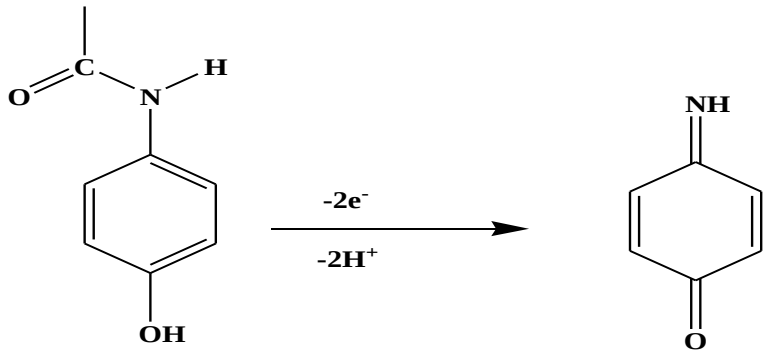


Fig. 9. Plot of cathodic peak potential (E_{pc}) versus pH at a scan rate of 100 mV s^{-1} using AGCE.

The linear dependence of the peak potential on pH was represented by the equation: E_{pa} (V) = - 0.041pH + 0.631 (R^2 = 0.9969) and E_{pc} (V) = -0.045pH + 0.632 (R^2 = 0.9917). The slopes of – 0.045 V/pH and -0.041V/pH suggests that the overall electrochemical process involves transfer of equal number of electrons and protons (scheme 4), which is in agreement with the reported literature [8, 9, 10]. Based on this finding, the most probable reaction mechanism for the oxidation of PAR phosphate buffer solution at AGCE shown below,



Scheme 4. Suggested oxidation reaction of paracetamol on AGCE.

4.2.2. Effect of scan rate on peak current and peak potential

The influence of varying scan rates on the electrochemical response of paracetamol at activated GC electrode was investigated by cyclic voltammetry. The effect of scan rate on the oxidation peak current of 0.1 mM paracetamol using AGC electrode in 0.1M PBS (pH 7.0) was studied by varying the scan rate from 20 - 300 mV s^{-1}. The resulting voltammogram as shown below in **Fig. 11,**

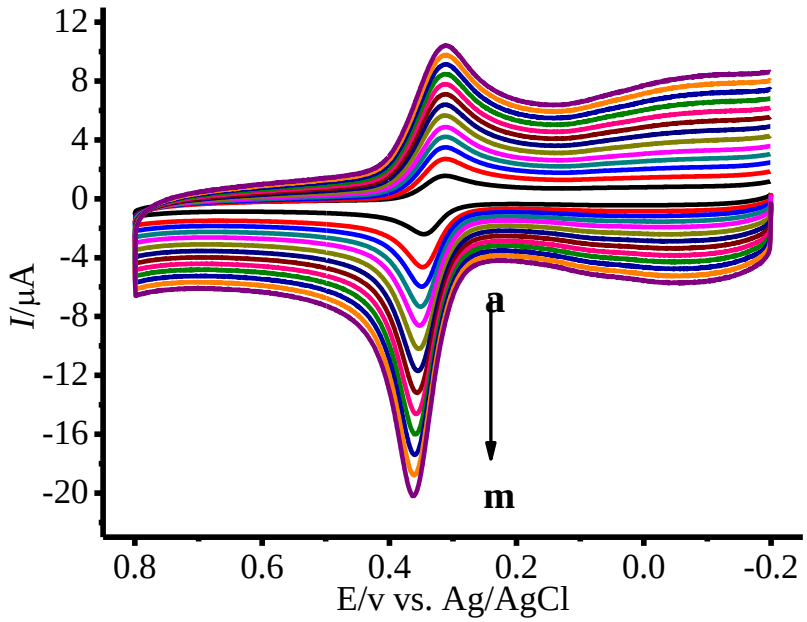


Fig. 10. Cyclic voltammogram of 0.1 mM PAR at different scan rates in the range (20, 40, 60, 80, 100, 125, 150, 175, 200, 225, 250, 275 and 300 mV s^{-1}) in 0.1M PBS (pH 7.0) at AGCE (Background subtracted).

The relation between peak current versus scan rate and square root of scan rate were drawn in **Fig.11** and **12** respectively.

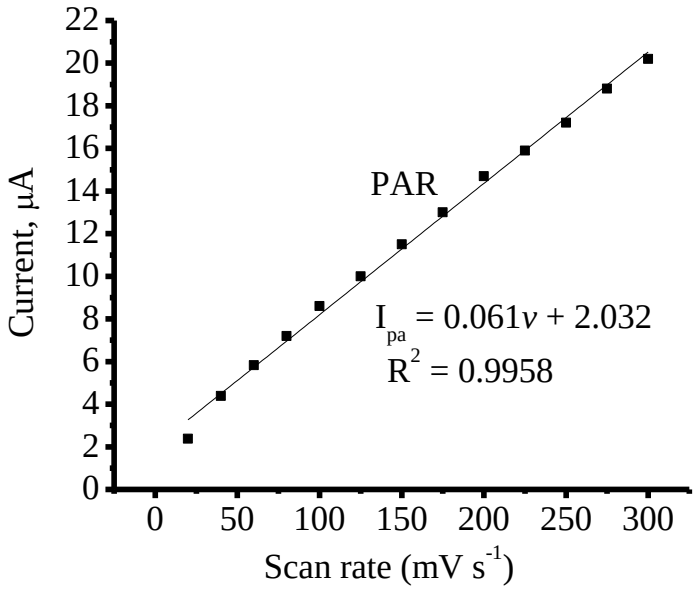


Fig. 11. Effect of variation of scan rate on the anodic peak current of 0.1 mM of PAR in 0.1 M of PBS at pH 7.0.

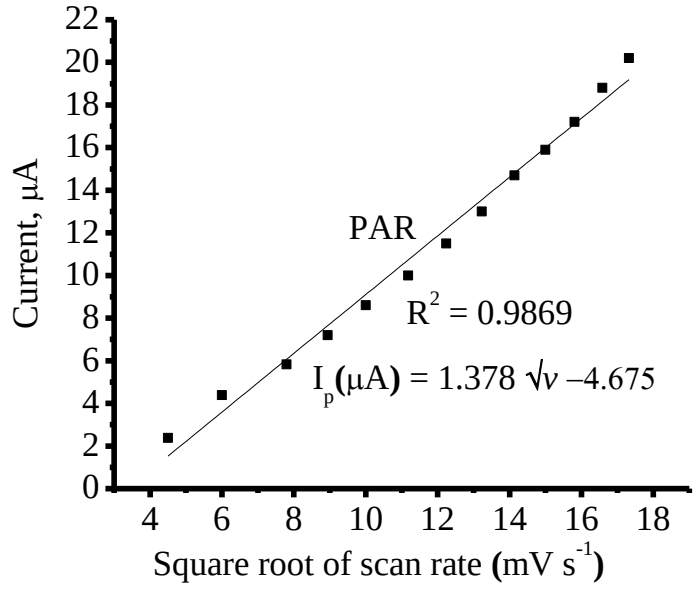


Fig. 12. The dependence of peak current of 0.1 mM of PAR on the square root of scan rate at AGCE in 0.1 M PBS at pH 7.0.

The linear equation of oxidation peak current on both scan rate and square root of scan rate as follows, I_{pa}= 0.061v+2.032 (R^2=0.998); and the oxidation peak current increased linearly as the square root of the scan rate, $\sqrt{v}$, I_{pa}= 1.37 $\sqrt{v}$ - 4.675 (R^2=0.9869).

The dependence of anodic peak current on the scan rate indicated that the electrode transfer reaction of paracetamol was adsorption controlled. Further evidence for non-diffusion behaviour of paracetamol was obtained, when the working electrode was switched to a medium containing only phosphate buffer solution after being in voltammetric measurements of paracetamol solution, voltammetric signal was observed [7].This is due to the interference of previous reaction products

4.3. Determination of kinetics parameters

The methods of cyclic voltammetry was used to determine the kinetics parameters such as transfer coefficient (α) and catalytic reaction rate constant (k_s) [39]. The electron transfer coefficient (α) can be calculated from the slope of the resulted curve of E_{pa} vs. Log *v* using equation:

$$E_{pa} = K + \frac{2.3RT \log v}{2(1-\alpha)n_{\alpha}F} \quad (3)$$

$$\text{Slope} = \frac{2.3RT}{2(1-\alpha)n_{\alpha}F} \quad (4)$$

Where α is transfer coefficient, n is the number electrons involved in the rate determining step, *v* is scan rate, R is gas constant and E_{pa} is peak potential.

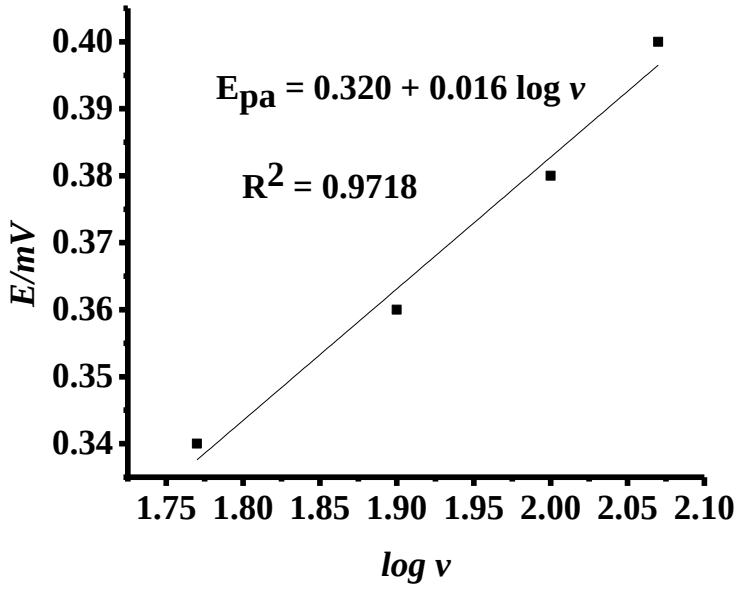


Fig. 13. Part of E_{pa} vs. log *v*.

Based on **Fig. 13** and equation (4), the value of transfer coefficient (α) was calculated as,

$$0.0168 = \frac{2.3RT}{2(1-\alpha)n_{\alpha}F} \qquad (5)$$

The value of transfer coefficient in this calculation was 0.121. This value indicates that when the electron transfer coefficient value is higher the deviation from reversible system. By calculating α from the slope of E_{pa} vs. log *v* curve, k_s can be determined from equation (5)

$$\text{Log } k_s = \alpha \log (1- \alpha) + (1-\alpha) \log \alpha - \log (\frac{RT}{nFv}) - \alpha (1-\alpha) \text{ n F} \frac{\Delta Ep}{2.3RT} \qquad (6)$$

Where α is electron transfer coefficient, k_s is heterogeneous electron transfer rate constant. The value of α was calculated as 0.121 and n = 2. By substituting the values in equation (6), k_s was calculated.

The heterogeneous electron transfer rate constant $k_s = 4.67 \times 10^{-2}$ s^{-1}. This shows that when the k_s value is high the AGCE can effectively promote the electron transfer between the solution and the electrode and when k_s is not high enough, the analyte peak passes over the electrode surfaces, its electrochemical reaction was slow and a broad peak was observed [39].

4.4. Differential pulse voltammetric investigation of PAR using AGCE

To further increase the sensitivity and lower detection limit, a more sensitive technique compared to cyclic voltammetry is differential pulse technique used to detect PAR at AGC electrode to evaluate calibration characteristics, validation (such as linearity, accuracy of real sample, limit of detection and limit of quantification).

Fig.14. shows the differential pulse voltammogram of 1.0×10^{-2} M paracetamol. Only one sharp peak was observed at 325mV, which indicates that paracetamol undergoes only one step electrochemical reaction at glassy carbon electrode when the potential was run in the positive direction, i.e., oxidation reaction at 325mV.

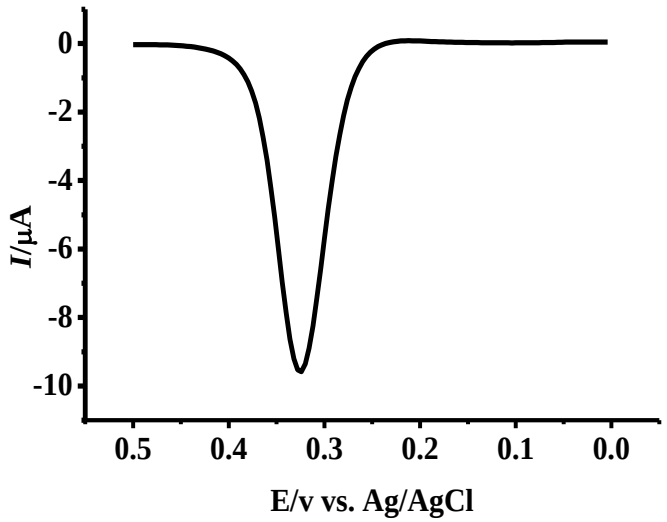


Fig.14. Differential pulse voltammogram of 0.1 mM PAR in 0.1M PBS (pH 7.0) at AGCE with a scan rate of 100 mV s^{-1} (Background subtracted).

As can be seen, the above voltammogram shows the peak current response in CV and differential pulse voltammogram was -7.10 and -9.66 μA respectively. This shows the peak current enhancement of paracetamol response was better in differential pulse voltammetric than cyclic voltammetry. So, differential pulse voltammetric was better peak current enhancement and good sensitive for the determination of paracetamol at AGCE.

4.4.1. Effect of concentration and detection limit

The effects of varying paracetamol concentration on the differential pulse voltammetric peak current response of paracetamol was studied at activated glassy carbon electrode. **Fig. 15** below shows DP voltammogram of PAR from 1 μ M - 60 μ M.

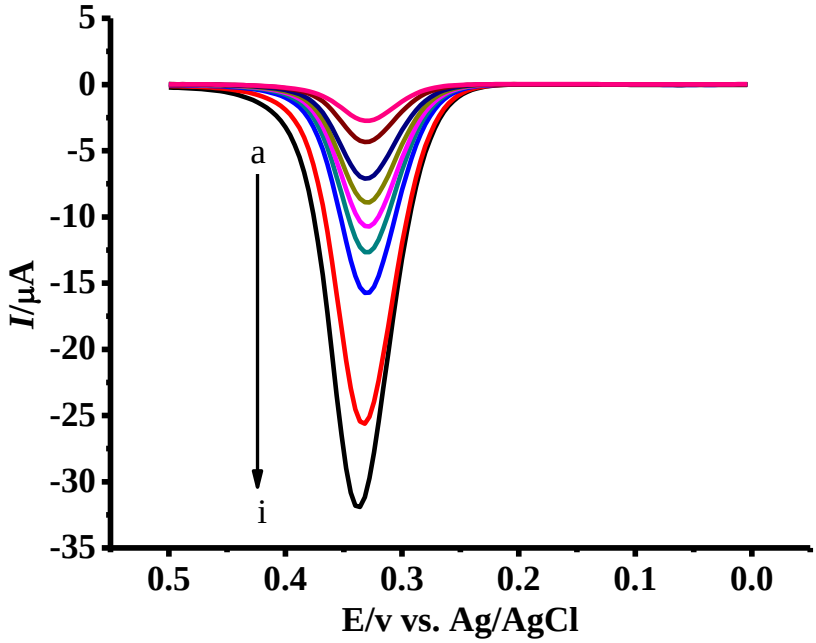


Fig. 15. Differential pulse voltammogram of PAR at different concentrations range (1, 2, 4, 6, 8, 10, 20, 40 and 60 μ M) in 0.1 M PBS pH 7.0 at the scan rate of 100 mV s^{-1} (Background subtracted).

The above voltammogram shows the oxidative peak current of paracetamol increases rapidly and linearly with increasing concentration from (1- 60 μ M). The resulting differential voltammogram consists of current peaks and the height of these current peaks is directly proportional to the paracetamol concentration [1].The enhancement of peak current on increasing paracetamol concentration in the above voltammogram is due to the presence of more ions in the solution which makes the flow of electrons easy [7]. The plot of differential pulse voltammetric peak current versus concentrations of paracetamol was found to be in the linear range of 8 - 60 μ M with correlation coefficient r^2 = 0.996 with the equation I_{pa} (μA) = 6.43 + 0.429 C (μ M).

The detection limit of PAR can be calculated by measuring the differential pulse voltammetry of activated GC electrode without PAR 8 times and calculate the standard deviations of 8 repeat measurements. The standard deviation of 8 measurements was 0.0115.The magnitude of detection limit calculated by using the formula; LOD = 3 δ /m, where δ represent the standard deviation of blank solution of 8 measurements and m represents the slope of the calibration curve. The detection limit was found to be 8×10^{-8} M.

The limit of quantification was calculated by the equation: LOQ = 10δ/m. The limit of quantification was found to be 2.6×10^{-7} M. The relative standard deviation (RSD) was calculated by standard deviation divided by the mean of 8 repeated measurement times 100. The relative standard deviation was calculated to be 1.02 %.

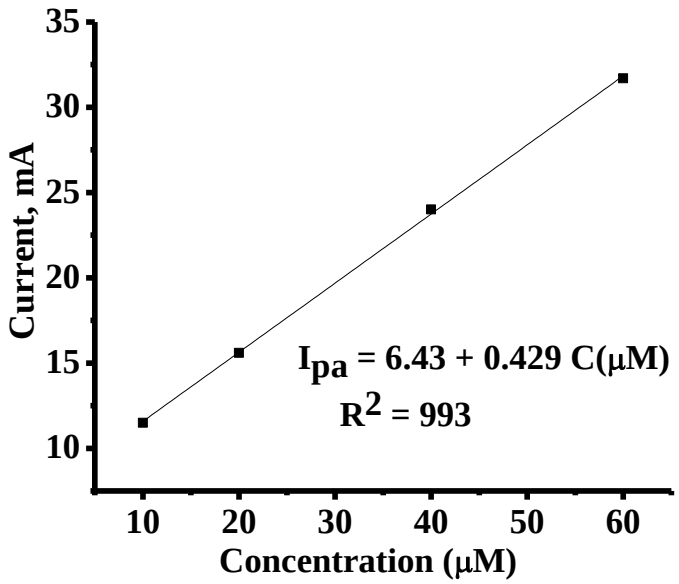


Fig. 16. Plot of peak current versus concentration.

As can be seen from the above fig**,** the peak currents of paracetamol concentration from 1to 6 μ M was lie outside the straight line. Therefore, the linear range extends only from 10 to 60 μ M paracetamol concentration.

4.5. Analytical application of the AGCE to pharmaceutical samples

4.5.1. Determination of paracetamol in tablets using DPV technique

In order to check the performance of the activated glass carbon electrode in real sample analysis, it was used to determine the concentration of paracetamol in tablets. So, a commercial pharmaceuticals paracetamol tablets (500 mg/tablet) sample was analyzed to evaluate the validity of the proposed methods. The sample obtained from the dissolution of tablets was diluted to 10 μ M solution of standard paracetamol sample. The concentration of paracetamol in tablets formulations was determined from the calibration curve plotted as standard concentration added versus peak current represented in equation (7).

$$y = mx + b \qquad (7)$$

Where y is peak current in μA, b is y-intercept in μA, m is the slope of the calibration curve and x is concentration in μM [1]. The concentration of PAR in tablet sample was then calculated to be x = 40.6 μM.

4.5.2. Determination of degree of recovery of paracetamol

To determine whether excipients in the tablets interfered or not, the accuracy of the proposed method was evaluated by recovery tests without spiking these samples and also on spiking known standard concentration of paracetamol in these tablets sample. The recovery results of paracetamol obtained by using DPV technique with AGC electrode, for all spiked and non spiked tablet sample have been calculated by using the concentration of spiked sample obtained from the calibration curve minus the concentration of non spiked sample divided by the concentration of the analytes added to the spiked portion (10 μM) times 100.

$$\% \text{ Recovery} = \frac{S - U}{A} \times 100$$

Where S represents the spiked sample paracetamol in μM, U represents un-spiked sample in μM and A represents the concentration of analyte added to the spiked portion (10 μM).

The %recovery of PAR in EPHARM tablets was calculated to be 105%, indicating that the AGCE could be successfully applied for PAR determination in tablets with a good recovery. The analysis of the obtained responses allowed concluding that the drug excipients do not significantly interfere with the proposed method. The amount of PAR on pharmaceuticals formulations of EPHARM was good agreement with the tablet manufacturer.

Table 2. Determination of paracetamol in formulation EPHARM tablets with AGCE.

Analyte	Concentration expected (μM)	Concentration found (μM)	Concentration difference (μM)
EPHARM	40	40.6	0.6
	50	51.1	1.1

4.5.3. Interference study on the behaviour of paracetamol

Paracetamol generally suffers from the interferences such as p-aminophenol, caffeine, ascorbic acid, uric acid and glucose. Hence, in this study a systematic study of interference due to the presence of uric acid only [12]. The interference UA was examined on AGCE on the determination of paracetamol. The oxidation potential of PAR is the same as that of UA, 0.3V and that of UA is very close at, 0.2 V. The voltammetric current response of successive addition PAR were recorded in **Fig. 17**, using AGC electrode in PBS (pH 7.0), containing equal amount of 0.1 mM PAR and 0.1 mM UA to check the selectivity of the method in the presence of interference.

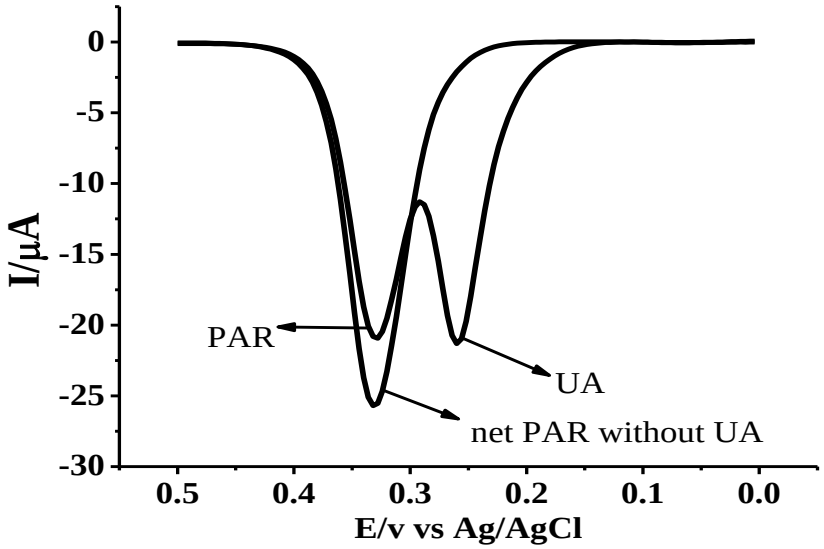


Fig.17. Differential pulse voltammogram of 0.1 M of PBS (pH 7.0) contains 0.1 mM PAR and 0.1mM UA using AGCE at a scan rate of 100 mV s^{-1} (Background subtracted).

As shown **Fig. 17.** two completely resolved peaks are observed at AGCE and the E_p of uric acid was observed at positive potential (~ 25 mV) with AGCE also the potential peak to separation of the two analytes were 90 mV. The well-defined peak of PAR was obtained at AGCE with good peak separation from UA [12]. Generally, the above voltammogram was observed that UA affect peak current for PAR compared with PAR without UA under the potential range used (i.e decrease the peak current and causes to the broadness of the peak of paracetamol), but do not affect the peak potential of paracetamol.

The percent changes in the peak current response of UA was 2.02%, suggested that UA do not significantly interfere in the determination of paracetamol.

4.5.4. Comparison of the proposed method with other methods

The electrochemical determination of paracetamol in this study is compared to other methods as summarised in table 3. As can be seen that the electrochemical determination of PAR in different electrode such as, nanogold modified carbon paste electrode [21], C_{60}-modified GCE [12], Poly (4-vinylpyridine/multi-walled carbon nanotubes modified GCE [8], GCE [13] and Poly (3, 4-ethylenedioxythiophene modified GCE [9]. This electrode were provides a reasonable analytical performance and good detection limit. But the activated glassy carbon electrode offers easy to activate with a potential and rapid electrode preparation compared to other electrodes.

Table 3. Comparison of the developed method with other modified electrodes reported in the previous literature.

Electrodes	Techniques	Linear dynamic Range	Detection limit	References
NiHCFMCPE	CV	5×10^{-4}-7.5×10^{-3} M	8.89×10^{-5} M	[7]
PEDOT/GCE	DPV	1.5 – 150 μ M	1.3 μ M	[9]
PolyAniBMCPE	DPV	0.1 – 0.8 μ M	1.179 μ M	[10]
C_{60}-modified GCE	DPV	0.05 -1.5 mM	0.05mM	[12]
GCE	DPV	4×10^{-6}- 1×10^{-4} M	3.69×10^{-7} M	[13]
GNMCPE	DPV	5×10^{-8}-2.7×10^{-4} M	1.46×10^{-8} M	[17]
SPGrE	CV	0.1-50 μ M	20nM	[19]
AGCE	DPV	8 - 60 μ M	8×10^{-8} M	This method

5. CONCLUSIONS

In the present study, an easily activated GCE was used to investigate the detailed electrochemical behaviour of paracetamol. The reported activated electrode significantly improved the electrochemical response of PAR and clearly demonstrates the excellent electro-catalytic properties of the AGCE toward the oxidation of paracetamol. Compared to other modified electrode, the potential activated glass carbon electrode was easily activated by applied potential and no need steps to activate. But the chemically modified GCE is the need of extra time through the consuming modification process which usually involves several steps to incorporate modifier to the substrate and also the costs. The electron transfer coefficient (α) and heterogeneous rate constant (k_s) were calculated from cyclic voltammetric response. The proposed method is clean, easy to set up (no need of special training) and furthermore, it does not require any expensive reagents apart from a simple buffer solution, and more time efficient. The activated glassy carbon electrode showed a linear response range between 10 – 60 μM and a detection limit of 8.0×10^{-8} M. The proposed method was applied for paracetamol determination in EPHARM commercial tablets with a recovery of 105%.

6. REFERENCES

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